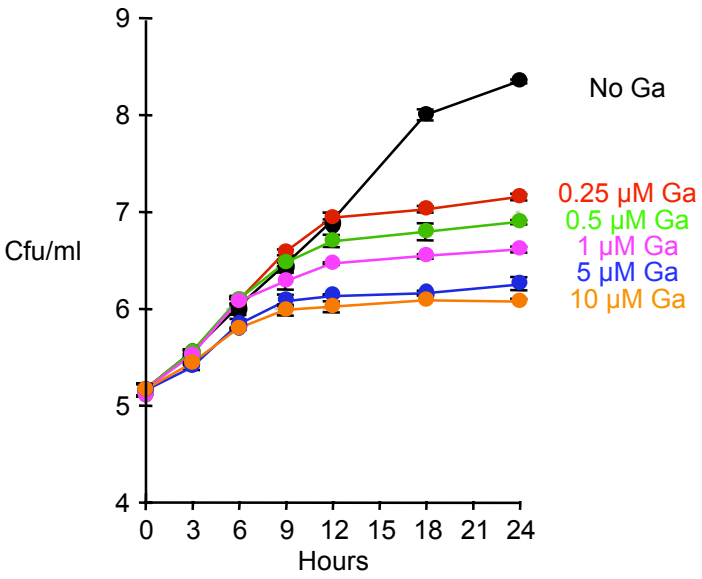

Fig S1

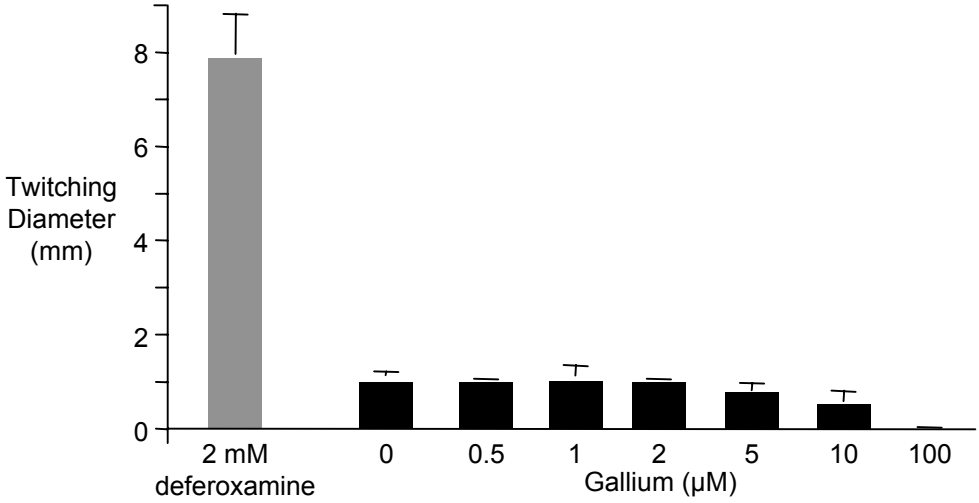

Fig S2

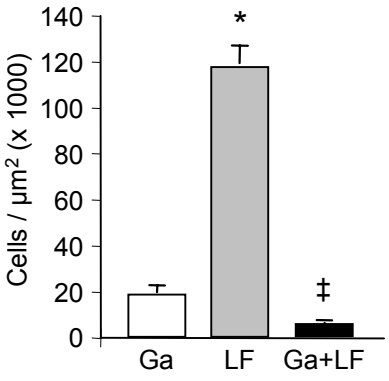

Fig S3

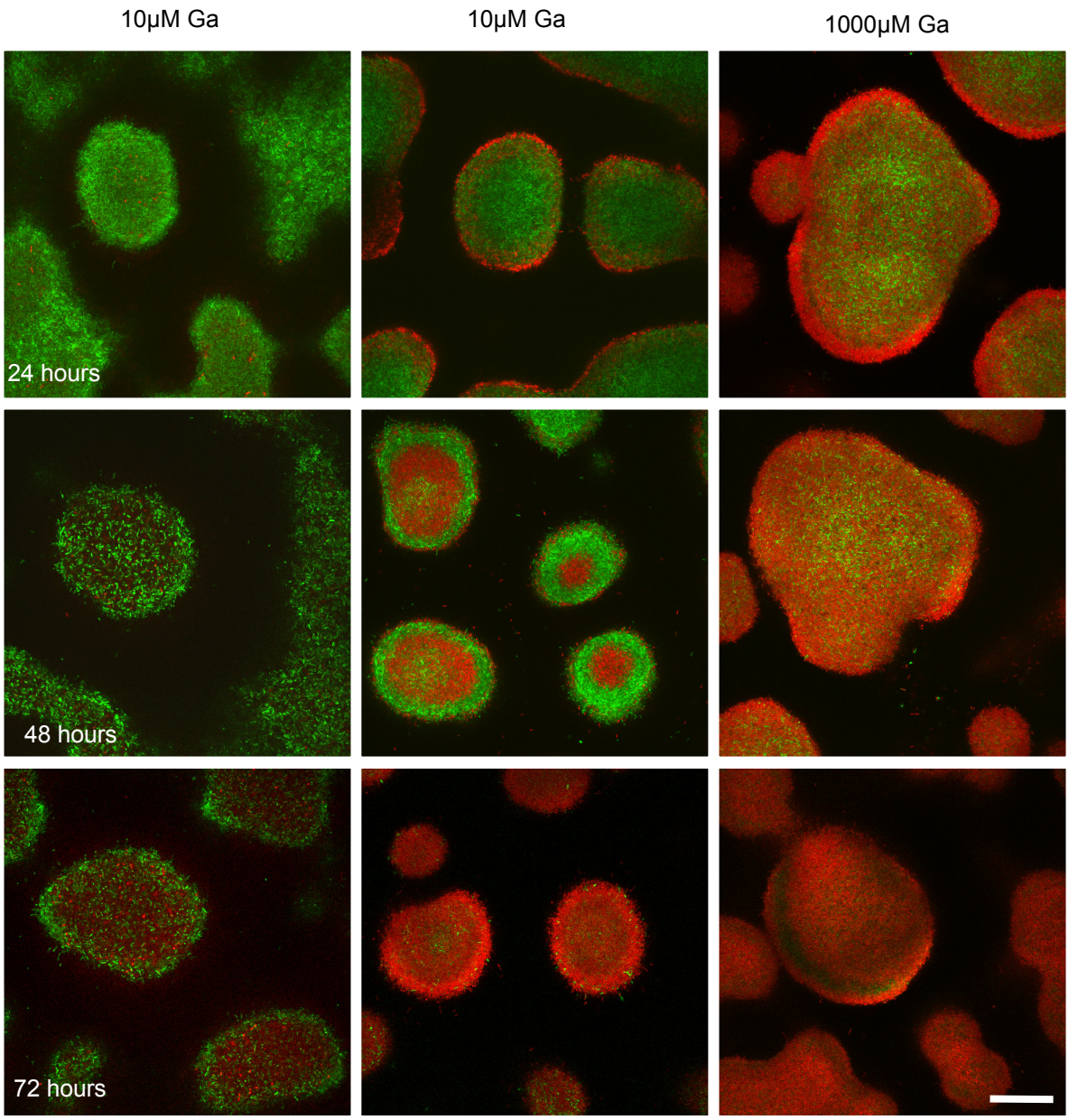

Fig S4

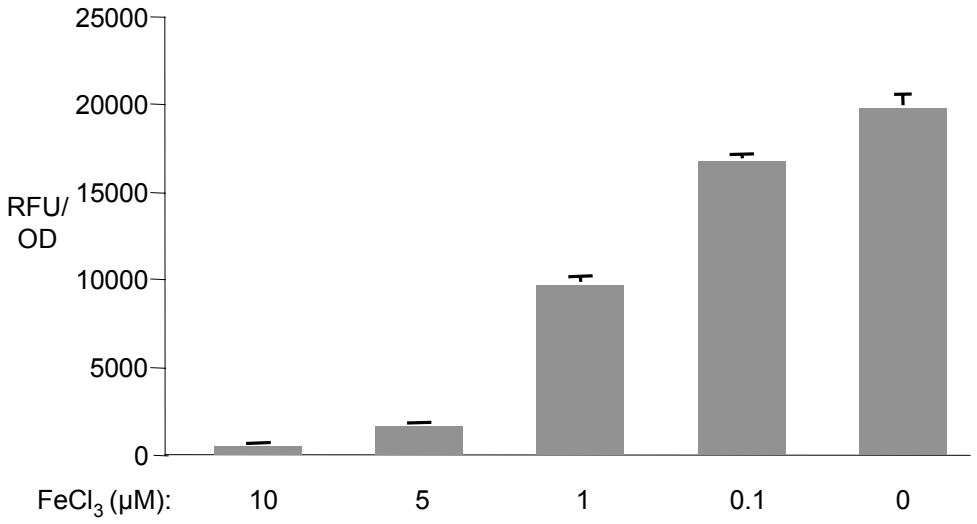

Fig S5

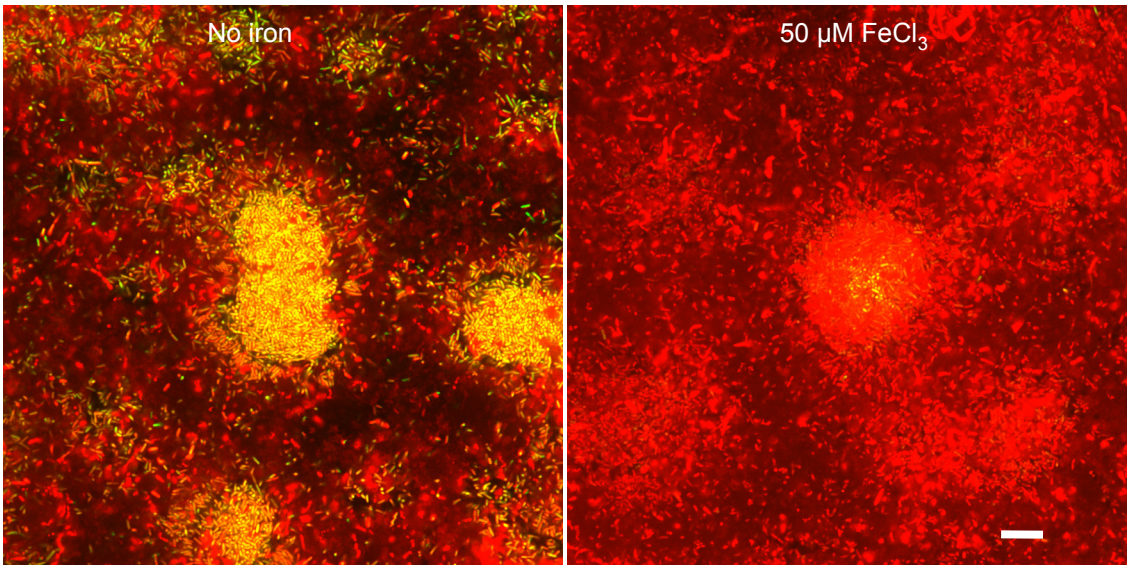

Fig. S6

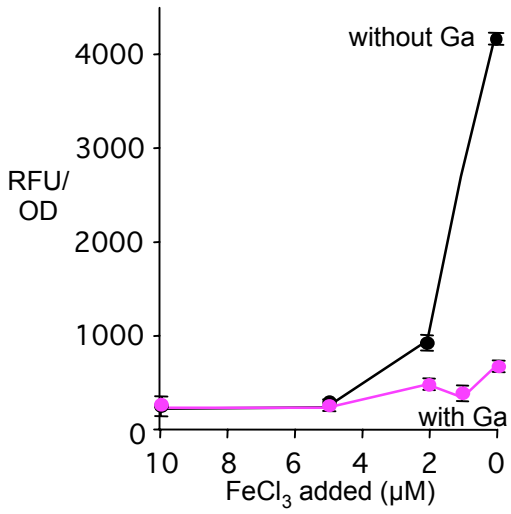

Fig. S7

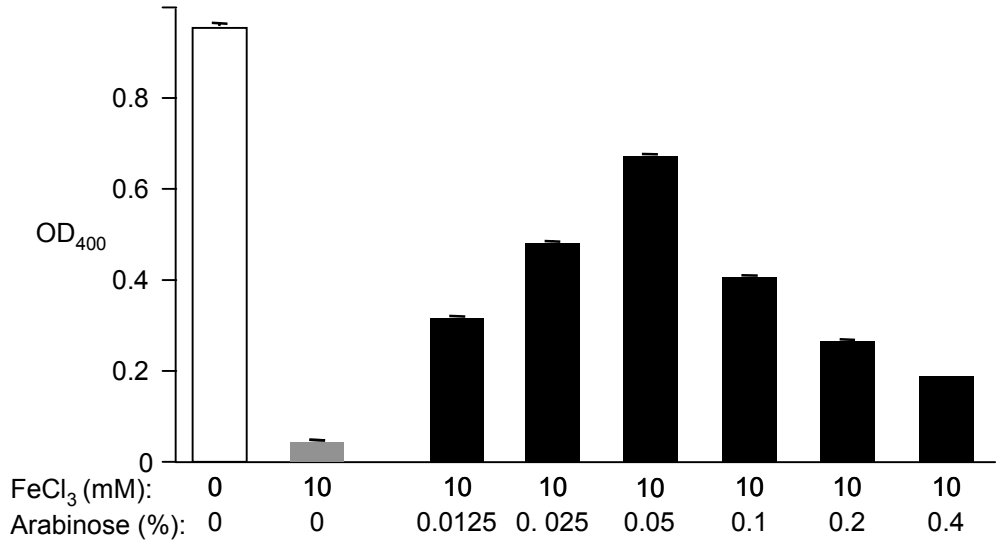

Fig S8a

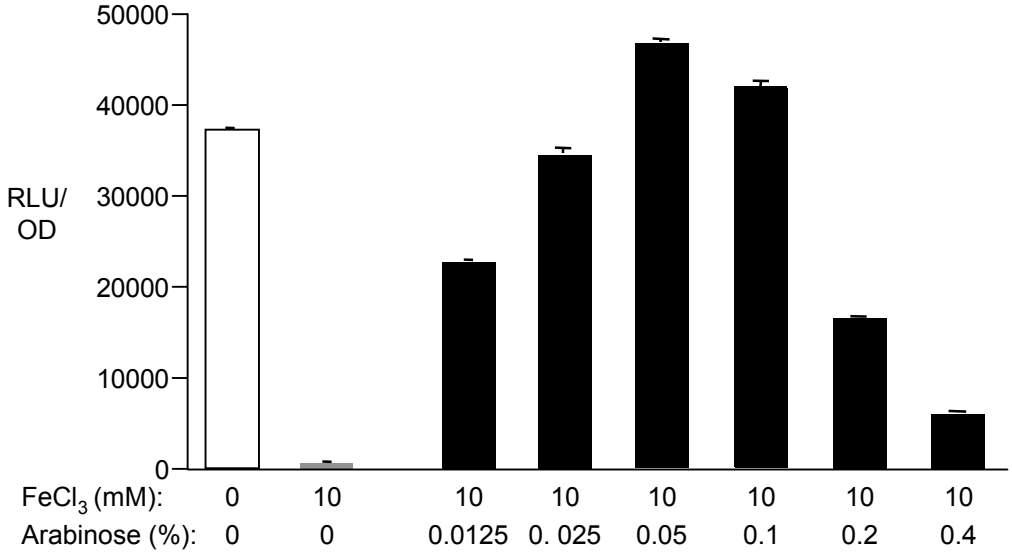

Fig S8b

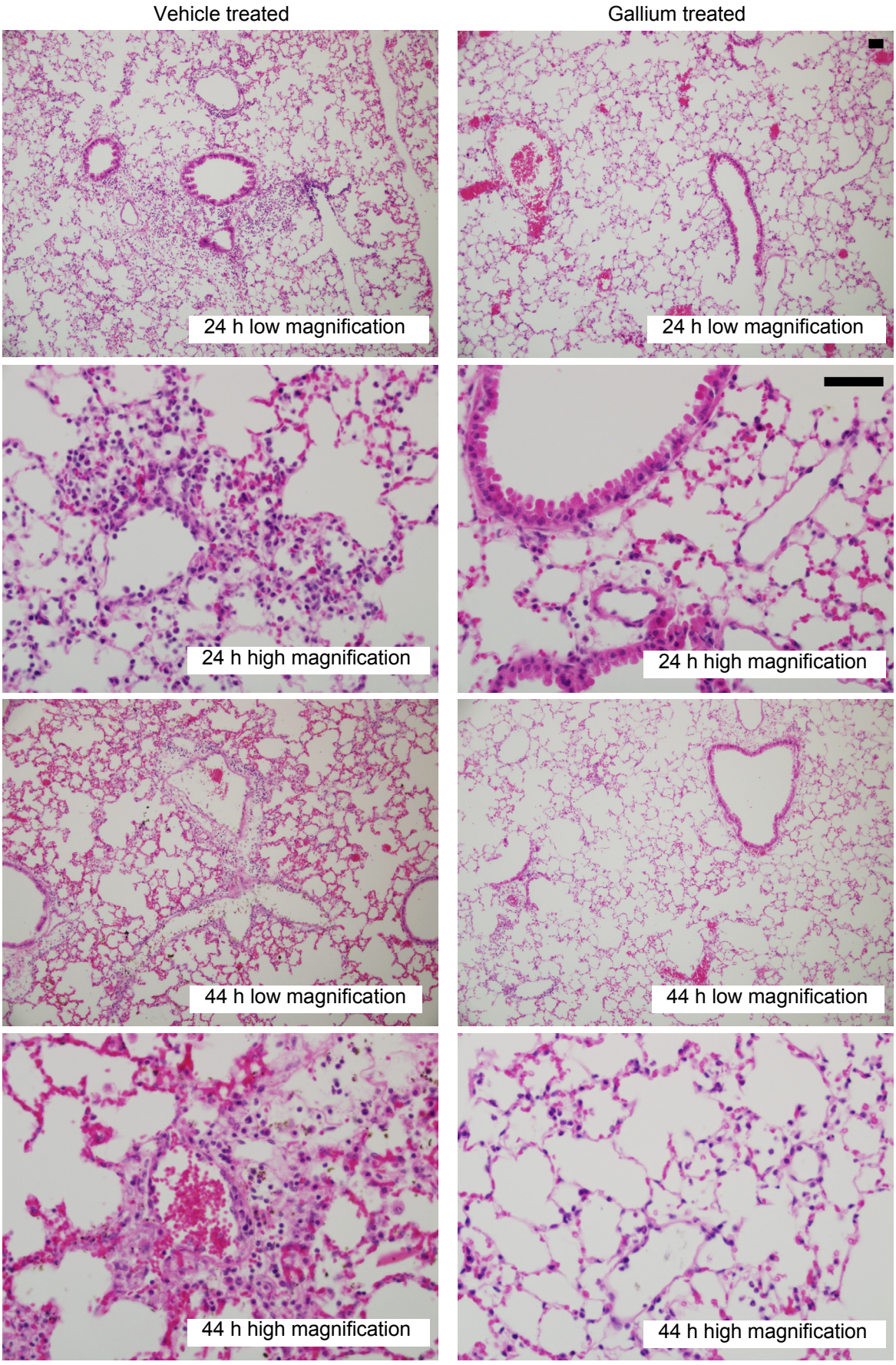
Vehicle treated
Gallium treated
24 h low magnification
24 h low magnification
24 h high magnification
24 h high magnification
44 h low magnification
44 h low magnification
44 h high magnification
44 h high magnification

Fig S9

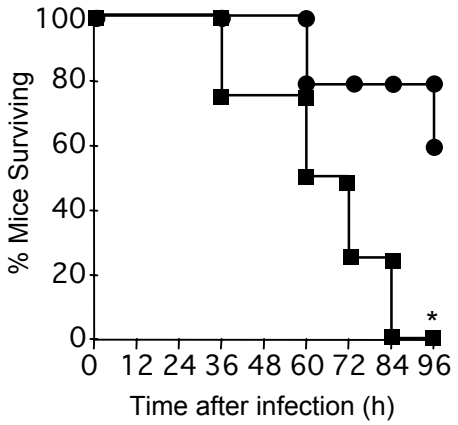

Fig. S10

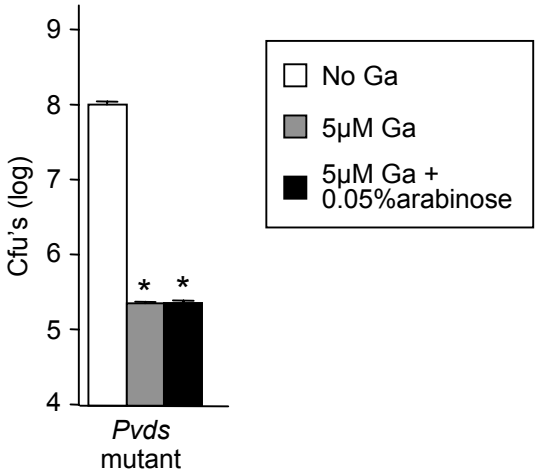

Fig. S11

Supplementary Figure Legends

Figure S1. Effect of Ga on *P. aeruginosa* growth at 25°C. $Ga(NO_3)_3$ inhibits *P. aeruginosa* growth in a concentration dependent manner. Data are the mean of 3 experiments; error bars indicate SEM.

Figure S2. Effect of the Fe chelator deferoxamine and Ga on the twitching motility of *P. aeruginosa*. Indicated concentrations of deferoxamine and $Ga(NO_3)_3$ were added to twitching motility plates and the twitching distance was measured after 3 days. Methods for twitching assay are described in (9).

Figure S3. Ga potentates the anti-biofilm effects of lactoferrin. GFP-labeled *P. aeruginosa* were grown in flow cells with biofilm medium containing Ga (1μM), lactoferrin (20 μg/ml), or lactoferrin (20 μg/ml) + $Ga(NO_3)_3$ (1μM). Twenty randomly selected confocal images each from 3 separate experiments were obtained after 5 days of growth, and the number of attached cells enumerated. Error bars are SEM; * indicates $P < 0.001$ vs. Ga treatment; ‡ indicates $P < 0.001$ vs. lactoferrin treatment.

Figure S4. Gallium kills established biofilms. Biofilms were grown using GFP labeled wild-type *P. aeruginosa* for three days in the absence of Ga, then treated with indicated concentrations of $Ga(NO_3)_3$ for 24, 48, and 72 h. Biofilms were then treated with propidium iodide (30μM) which stains dead cells red. Images are top-down views (X-Y plane); scale bar, 50 μm. Results are representative of 4 separate experiments. Also see Fig. 2.

Figure S5. Iron limitation stimulates *pvdA* expression. A PAO1 strain containing the *pvdA-gfp(S)* reporter was incubated with indicated concentration of $FeCl_3$. Expression of *pvdA* increased as Fe concentrations were lowered.

Figure S6. Evidence for iron starvation in the central biofilm regions. *P. aeruginosa* constitutively expressing red florescent protein and containing the *pvdA*-gfp reporter (which fluoresces green during Fe limitation) were grown in biofilms for three days ± $FeCl_3$ (50μM). In the absence of added Fe, green florescence can be seen in the central biofilm regions indicating Fe limitation. Iron addition reduced the size of the central Fe-starved (green) region. Images are top-down views (X-Y plane); scale bar, 50 μm. All images were taken with identical confocal microscope settings, and results are representative of 3 separate experiments.

Figure S7. Gallium represses pyoverdine gene expression normally induced by Fe starvation. Wild type *P. aeruginosa* was grown in BM-2 medium with indicated concentrations of $FeCl_3$ in the presence and absence of Ga (2 μM). Ga treatment blocked the increase in pyoverdine gene expression normally induced by Fe starvation. Gallium also blocks pyoverdine biosynthesis induced by Fe starvation (Fig. 6c).

Figure S8. Induced expression of *pvdS* increases pyoverdine production. The PA01 ara-*pvdS* strain containing the *pvdA-gfp(S)* reporter was incubated with indicated concentrations of arabinose and $FeCl_3$. Addition of 10 μM Fe suppressed secretion of pyoverdine (**a**) and expression of *pvdA-gfp(S)* (**b**). Arabinose-induced *pvdS* expression increased secretion of

pyoverdine (**a**) and *pvdA-gfp(S)* expression (**b**) even in the presence of Fe. Pyoverdine measurements in culture supernatants were performed as described in (45). Expression of *pvdA* was measured by incubating 10^6 planktonic PAO1 *pvdA-gfp(S)* bacteria in BM2 with $FeCl_3$ at 37ºC for 24 h. The OD_{590} and the relative fluorescent units (10 reads each) were measured using a GENiosPro (Tecan) and averaged.

Figure S9. Gallium reduces histological evidence of lung injury in the acute pneumonia model. Infections were established with PA103 and animals were sacrificed at the indicated times. PBS (10 ml) was perfused through the right ventricle and then the lungs were removed intact. The trachea was intubated and instilled at 8 cm of pressure with PBS±4% paraformaldehyde and 0.2% gluteraldehyde. Lungs were embedded in paraffin and serially sectioned. After staining with hemotoxylin and eosin, 3 sections were obtained from the apical region, from the midregion and from the basal region of the lung of each animal. Images shown are representative of 9 sections taken from the lungs of three different mice treated with Ga or vehicle.

At 24 h, vehicle treated lungs show areas of airspace pneumonia and perivascular inflammation. High magnification views show moderate neutrophilic airspace infiltrates and areas of pulmonary capillary congestion. Ga treated lungs predominately show mild interstitial edema. At 44 h vehicle treated lungs show increased pulmonary vascular congestion, and neutrophilic airspace inflammatory infiltrates. Ga treated lungs show only mild perivascular inflammation, and small areas of pneumonia.

Figure S10. *P. aeruginosa* strain PA01 ($5x10^6$ bacteria) were administered to mice by the intratracheal route. Mice were treated with inhaled Ga (or vehicle alone) 1 h before and 3 h after

infection. Inhalation treatment was achieved by placing a 50 μl drop of concentrated $Ga(NO_3)_3$ (250 mm) or Ga-free vehicle on the nares of the mice. Because mice are obligate nose breathers, some of the drop is inhaled; * indicates P< 0.04 vs. vehicle control.

Figure S11. Inactivation of *pvdS* somewhat enhances the inhibitory effects of Ga. The *P. aeruginosa pvdS* mutant was grown in biofilm medium ± 5 μM Ga and 0.05% arabinose (arabinose was present from the beginning of the experiment). The *pvdS* mutant was somewhat more sensitive than the wild type (compare to Fig. 7a). Results are the mean of 3 experiments; error bars are SEM; * indicates P <0.001 vs. untreated control.

Supplementary Material

Table S1. Other gene expression changes induced by gallium treatment.

PA ORF#	Gene	Fold change +Ga	Annotation[a]	Induced by low Fe (33)
Cytochrome oxidase				
PA0105	coxB	3.5	cytochrome c oxidase, subunit II	No
PA0113		3.0	probable cytochrome c oxidase assembly factor	No
PA1317	cyoA	-3.4	cytochrome o ubiquinol oxidase subunit II	Yes
Nitrate reductase				
PA0517	nirC	-5.5	probable c-type cytochrome precursor	No
PA0518	nirM	-4.0	cytochrome c-551 precursor	No
PA0519	nirS	-3.0	nitrite reductase precursor	No
Glucose transport and metabolism				
PA2322	gnuT	-6.6	gluconate permease	No
PA3181	edaA	-4.3	2-keto-3-deoxy-6-phosphogluconate aldolase	No
PA3182	pgl	-3.8	6-phosphogluconolactonase	No
PA3188	gltG	-3.9	probable permease of ABC sugar transporter	No
PA3189	gltF	-3.0	probable permease of ABC sugar transporter	No
PA3191		-6.2	probable two-component sensor	No
PA3192	gltR	-4.8	two-component response regulator GltR	No
PA3193	glk	-3.9	glucokinase	No
Glycolate metabolism				
PA5352	glcG	4.7	conserved hypothetical protein	No
PA5353	glcF	3.9	glycolate oxidase subunit GlcF	No
PA5354	glcE	4.0	glycolate oxidase subunit GlcE	No
PA5355	glcD	6.3	glycolate oxidase subunit GlcD	No
Fatty acid metabolism				
PA2862	lipA	4.1	lactonizing lipase precursor	No
PA2863	lipH	5.8	lipase modulator protein	No
PA4813	lipC	4.4	lipase LipC	No
PA1771	estX	-3.4	probable esterase/lipase	No

Supplementary Material
Continued on next page

Table S1. *continued*

PA ORF#	Gene	Fold change +Ga	Annotation[a]	Induced by low Fe (33)
Ribosomal proteins				
PA4250	rpsN	-3.4	30S ribosomal protein S14	No
PA4255	rpmC	-3.6	50S ribosomal protein L29	No
PA4257	rpsC	-3.0	30S ribosomal protein S3	No
PA4260	rplB	-3.2	50S ribosomal protein L2	No
PA4273	rplA	-3.0	50S ribosomal protein L1	No
PA4671	rplY	-4.1	probable ribosomal protein L25	No
Tryptophan synthase				
PA0035	trpA	-3.4	tryptophan synthase alpha chain	No
PA0036	trpB	-3.7	tryptophan synthase beta chain	No
Arsenic resisntant				
PA2279	arsC	4.2	ArsC protein	No

Continued on next page

Table S1. *continued*

PA ORF#	Gene	Fold change +Ga	Annotation[a]	Induced by low Fe (33)
Other or unknown functions				No
PA0034		-3.9	probable two-component response regulator	No
PA0382	micA	-3.0	DNA mismatch repair protein MicA	No
PA0693	exbB2	3.3	transport protein ExbB2	No
PA0896	aruF	-4.2	arginine/ornithine succinyltransferase AI subunit	No
PA1190	yohC	3.3	conserved hypothetical protein	No
PA2024		3.2	probable ring-cleaving dioxygenase	No
PA2033		-3.4	hypothetical protein	Yes
PA2079		3.2	probable amino acid permease	No
PA2445	gcvP2	-3.6	glycine cleavage system protein P2	No
PA3500		3.2	conserved hypothetical protein	No
PA3504		3.4	probable aldehyde dehydrogenase	No
PA3655	tsf	-3.3	elongation factor Ts	No
PA3780		-3.2	hypothetical protein	No
PA4149	acoX	3.1	conserved hypothetical protein	No
PA4480	mreC	-3.4	rod shape-determining protein MreC	No
PA4673	ychF	-3.3	conserved hypothetical protein	No
PA4680		3.3	hypothetical protein	No
PA4681		3.1	hypothetical protein	No
PA4981		3.0	probable amino acid permease	No
PA5118	thiI	-4.1	thiazole biosynthesis protein ThiI	No
PA5372	betA	4.0	choline dehydrogenase	No
PA5375	betT1	4.6	choline transporter BetT	No
PA5504		-3.2	probable permease of ABC transporter	No
PA5558	atpF	-3.0	ATP synthase B chain	No

[a] Gene name, number, and annotation are from the Pseudomonas genome project (http://www.pseudomonas.com).

Table S2. Bacterial strains and plasmids

Strain or plasmid	Relevant characterisics	Reference or source
Strains		
PA01	Wild type strain, PA01 (obtained from B. Iglewski)	(9, 48)
PA01-GFP	PA01 constitutively expressing GFP plasmid pMRP9-1	(9, 48)
PA01_*PvdA*	Unmarked *pvdA* mutant	(11)
PA01_*PvdD_PchEF*	Unmarked *pvdD* and *pchEF* mutant	(49)
PA01_*PvdS*	Insertion-deletion *pvdS* mutant Gm^R	(50)
PTL5032	PA01 transposon *pchA* mutant (see (a))	(51)
PTL14263	PA01 transposon *fecA* mutant (see (a))	(51)
PA01 *pvdA-gfp(S)*	PA01 with a *pvdA-gfp(S)* (short 1/2 life) fusion inserted at *attB*, Tet^R	this study, (52)
PA01 *pvdA-gfp*	PA01 with a *pvdA-gfp* (long 1/2 life) fusion inserted at *attB*, Tet^R	(11)
P. aeruginosa clinical isolates	Obtained from chronically infected CF patients	(see (b))
Plasmids		
pMRP9-1	Constitutive *gfp* plasmid, $Carb^R$	(48)
pUCP18	Escherichia-Pseudomonas shuttle vector, Amp^R	(53)
pJN105	araC-P_{BAD} cassette cloned in pBBR1MCS-5, Gm^R	(53)
pMJT1	araC-P_{BAD} cassette of pJN105 cloned in pUCP18, Amp^R ($Carb^R$)	this study, (54)
pMJT1-*pvdS*	araC-P_{BAD} cassette-PvdS fusion in pUCP18, Amp^R ($Carb^R$)	this study
pUCP18-*rfp*	mRFP cloned in pUCP18, Amp^R ($Carb^R$)	this study, (55)
Mini-CTX-lacZ	Integration-proficient vector for chomosomal insertion at the *attB* site, Tet^R	(56)
pMH489	Promotorless *gfp* (short 1/2 life) in pUCP18, Amp^R, Gm^R, Chl^R	(52)
pMiniCTX-pvdA-*gfp(S)*	*pvdA-gfp* (short 1/2 life) transcription fusion in mini-CTX-lacZ, Tet^R	this study

(a) The *P. aeruginosa* PA01 pyochelin mutant (*pchA*) and ferric citrate receptor mutant (*fecA*) (PTL5032 and 14263) were obtained from the *P. aeruginosa* transposon mutant library at the University of Washington (51). The locations of transposons were confirmed by PCR. In

studies using these mutants we utilized the parent PA01 from the University of Washington as the control and found it indistinguishable from PA01-Iglewski. (b) *P. aeruginosa* isolates from chronically infected CF patients were obtained from collections at the University of Washington (provided by J. Burns, S. Moskowitz and M. Olsen) and Columbia University (provided by L. Saiman). Abbrevations: Amp^R, ampicillin resistance; Tet^R, tetracycline resistace, Gm^R, gentamycin resistance; Chl^R, chloramphenicol resistance; $Carb^R$, carbenicillin resistance; gfp, green fluorescent protein; mRFP, monomeric red fluorescent protein.

Construction of plasmids and *P. aeruginosa* mutants.

The arabinose inducible *pvdS* construct was made by cloning araC-P_{BAD} of pJN105(54) into pUCP18(53) to generate pMJT1. A 587bp fragment containing *pvdS* was cloned into pMJT1 (see (57) for annotation) to generate pMJT1-*pvdS*. The constitutive red fluorescent protein (*rfp*) plasmid consisted of monomeric *rfp* (55), cloned in to pUCP18 (53). The short 1/2 life *pvdA-gfp(S)* fusion strain was constructed by cloning the *pvdA* promoter into pMH489 (52), which carries a promoterless short half-life *gfp* and transferring to PA01 by conjugation.